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The Stomata of Bluegums (*Eucalyptus* spp.)

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Abstract

Measurements of the sizes of the stomata of juvenile and adult leaves of Tasmanian blue-gum (*Eucalyptus globulus* Labill.) made over a long period and in diverse countries are collated. It is shown that in *E. globulus*, the sizes are independent of locality and whether the trees are natural or cultivated, that is almost certainly they are genetically determined. The stomata of adult leaves of different species of blue gums are larger, sometimes as much as twice as large, as those of the juvenile leaves. The upper surface of the juvenile leaves of three of the blue gum species bears few or no stomata and the internal structure of the (bifacial) leaf reflects this. The (unifacial) adult leaves always have stomata on both surfaces. The large adult/juvenile stomatal size ratio appears to be unique to bluegums since it does not occur in a range of other species investigated.

Introduction

Alhough Vesque (1882) is usually credited with drawing attention to the usefulness of studies of leaf anatomy to systematics Mueller (1883) was already aware of and using features of leaf anatomy in his research. He wrote: "By the aid of the microscope we may yet hope to be able to obtain characteristics of diagnostic value from the anatomy of leaves sufficiently positive to recognise ordinal and even perhaps generic groups..." "I was enabled, for instance, to demonstrate the existence of Epacridae in New Guinea from the microscopic comparison of the leaf epidermis of a species, brought from thence without flowers or fruits, with the very peculiar cuticle of many Epacridae easily recognized microscopically." Mueller was the first to investigate the microscopic features of the leaf epidermis of eucalypts. In his Eucalyptographia (1879–1884) he illustrated the stomata of 39 species. He not only discussed differences in sizes of the stomata but also the differences betweeen species in stomatal frequency (number per unit area of leaf surface). His younger contemporary, J.H. Maiden (1909–1928) referred (Vol. 1, p. 9) to Mueller's tentative classification based on the differences in distribution of stomata on the two leaf surfaces, but dismissed it, without citing supporting evidence, as "not reliable." Beyond this Maiden makes no further reference at all to stomata. Nevertheless, he does remark (*ibid*) "The anatomical characters of the leaves of *Eucalyptus* offer, however, much room for research."

In carrying out the statistically elaborate work for his 1973 doctoral thesis on Geographical variation in *Eucalyptus globulus* Labill. Kirkpatrick collected materials of bluegums, as the group of species to which *E. globulus* belongs is known, throughout south-eastern Australia.

His two papers on the topic (Kirkpatrick 1974, 1975) divide the group into four subspecies of which *E. globulus* proper is almost entirely Tasmanian with some outliers in southern Victoria, and *E. maidenii* F.Muell. is almost entirely restricted to the seaward slopes of south-western New South Wales. I differ from him in what follows in treating the entities he regards as subspecies as species in their own right (cf. also Chippendale 1988). The literature cited by Kirkpatrick is almost exhaustive of that on the bluegums: but there still remain some aspects of the bluegums, including some older literature, neither mentioned nor discussed. One of these aspects goes back to the work of Mueller.

It concerns the stomata of *E. globulus*. To Mueller, that name covered all the species (or subspecies) of bluegum now recognised (except *E. maidenii*) so it is difficult to be sure that his work on stomata really concerned only the Tasmanian species. Nevertheless, the illustration to his description of the species (Mueller 1879–84) is, as far as one can tell, true to the concept of *E. globulus* put forward by Labillardière in 1800 and 1806. Nevetheless, whereas *E. globulus* (in Labillardière's sense) has inflorescences each consisting of a single flower, an unnumbered picture of a three-flowered inflorescence is included in the Plate (Decade 6) without any comment in the legend. I think that Mueller would have been cautious enough to realise the possility of his specimen not being *E globulus sensu stricto.*

In constructing Table 1 I was unable to trace two references to *E. globulus* in early pharmacological journals, referred to by Maiden (1909–1920, Vol 1, p 7) which might contain measurements of stomata. Lacking funds to collect for my work, materials from the "wild," I requested, but was unable to obtain, access to Kirkpatrick's specimens but they had been returned to the collector. However there are plantings of most species of

Table 1. Stomatal size* and frequency in *E. globulus*

Author		Juvenile			Adult	
		Size µm	Frequency mm^2		Size µm	Frequency mm^2
Mueller	upper		70	upper	60	
	lower	132		lower	51	87
Briosi	upper			upper		
	lower	40–60	162	lower	80–100	59
Johnson	upper			upper	53	86
	lower	29	83.8	lower	54	86
Carr and Carr (1)	lower	27	142	lower	53	
Carr and Carr (2)	upper			upper	(na)	
	lower	26.5	191	lower	(na)	
Carr and Carr (3)	lower	27.6	127	lower	53.3	
Carr and Carr (4)	lower	35.4		lower	58.3	
Maiden & Mansfield (1968)	lower	31				
Ridge, R.W. (1980)	upper			upper	56	129
	lower			lower	57	63.6

*The size measurements in all Tables are of stomatal length (micrometres) (means of 30 determinations) .

bluegums in the Canberra area (Pryor 1951) and I turned to these for materials. We did have some specimens of *E. globulus* obtained from A.K. Cameron.

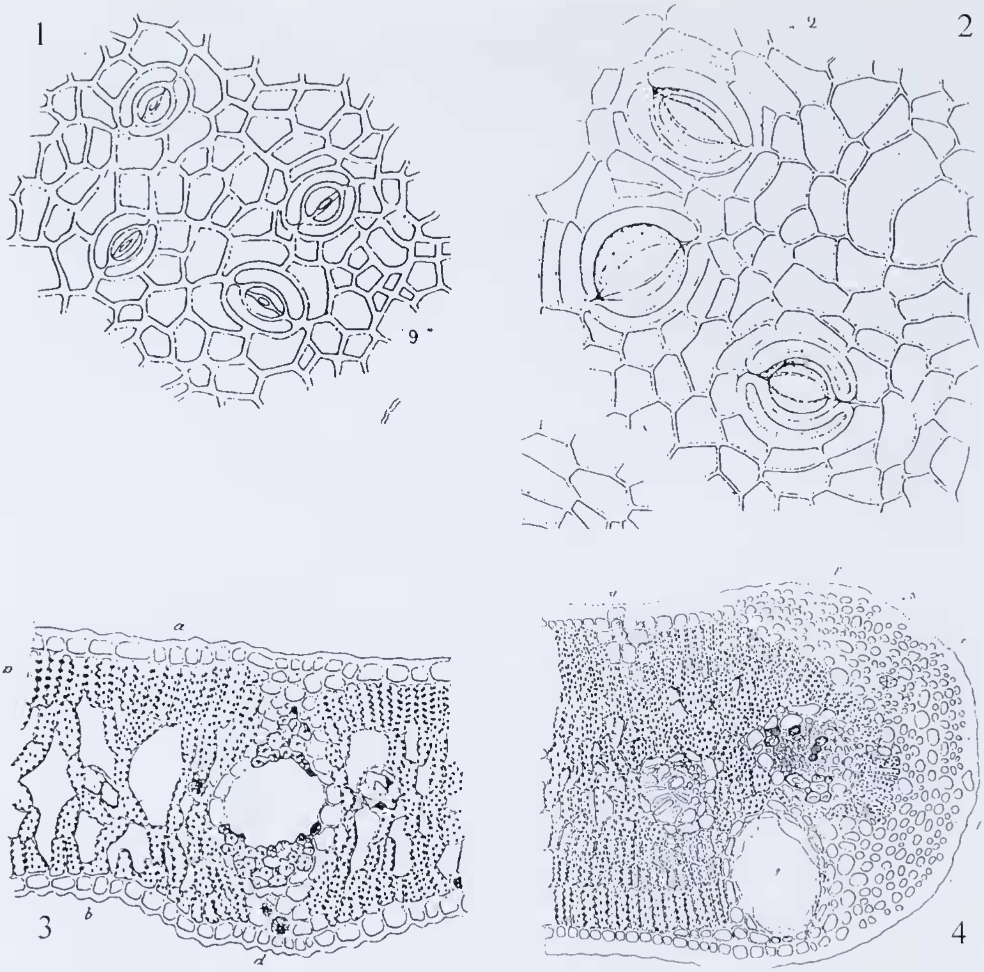
Subsequent to Mueller's research on *E. globulus* a monograph appeared in Italy on the leaf of *Eucalyptus globulus* which included a number of measurements and drawings of stomata (Briosi 1892). Since then a number of other measurements have been made, published or not, on the stomata of bluegums including *E. globulus*, which (in its narrow sense) (*contra* Kirkpatrick) is not widely grown on the mainland of Australia but is or was widely grown abroad. However, in the last few years, extensive plantings of *E globulus* have been made for timber and woodchips in southern and south-western Western Australia and in western Victoria.

Table 1 shows three remarkable things. One is the extrordinary consistency of the measurements on a single species, especially of the stomatal lengths over more than one hundred years and in places separated by the diameter of the earth, and irrespective of seed provenance. This attests to inherent that in *E. globulus* stomatal length is under strict genetical control. The only aberrant data are those of Briosi, which are twice those of all the other observers. The second remarkable thing is that in all but one case (Mueller) there were no stomata recorded on the upper side of the juvenile leaves. The third, is that in all cases the stomata on the lower side of the juvenile leaves were about or just under one half the length of those of the adult. Omitting Briosi's measurement the mean of the juvenile stomatal lengths is 29.4 μm with a standard deviation (SD) of 3.06; that of the adult stomata 54.6 SD 1.95. Thus the ratio of the two means is 1.86. This is also the case in the measurements of Briosi, which leads me to believe that his measurements suffer from a systematic error, perhaps in the calibration of his instruments. If his magnifications (given as ×300) were actually ×450 (as were Mueller's) the adult stomata illustrated in Briosi's Plate III Fig 2 would be 55 μm, not 80 to 100 as stated.

The Carr and Carr measurements were from specimens collected by A.K.Cameron and A.M.Gray in Tasmania. They had no stomata (Carr and Carr 2 & 4) or very few (1 and 3) (less than 1 mm^2) on the upper surface of the juvenile leaves; another specimen from Flinders Island, Bass Strait had none. The juvenile leaves of Mrs Carr's undated and unnumbered specimen from Squeaky Bay, Victoria, about as close a locality as one can get to Tasmania on the mainland of Australia, also lacked upper surface stomata. On the other hand, a specimen collected (as '*E globulus*') by Cameron in Toorak, Melbourne, Victoria, had stomata on both surfaces of the juvenile leaves, with a frequency of 31 (upper) and 42 (lower) mm^2. Johnston (1926) recorded "a few" stomata along the midrib on the upper surface of the juvenile leaves, evidently too few to record their frequency (*c.f.* Carr and Carr 1 & 3). The material she used was a plant grown in a greenhouse at Manchester. The material of Meidner and Mansfield (1968) was also a seedling grown in an English greenhouse. Mueller, whose concept of the species *E. globulus* was extremely wide (as already mentioned), found stomata on the upper surface of the juvenile leaves, reported by none of the other authors (but *c.f.* the Toorak specimen of Cameron). One cannot therefore be certain that Mueller's data refer to *E.globulus* in the narrower, modern sense. The size reported in Table 1 is from a drawing, together with its magnification, of a single stoma.

The stomata of the juvenile leaves of *E.globulus* (in the narrow sense) are therefore essentially restricted to the lower surface. This has implications for the internal structure of the leaf, which differs in this from the adult leaf which, having stomata on both sides, has also a layer or layers of palisade parenchyma below each leaf surface (Figs. 1–4). This, of course was already known to Briosi (1892: 106–107) but has escaped the notice of subsequent writers (e.g. Maiden. 1909–1920 (Vol 1: 7; 6: 287); Penfold & Willis 1961), who cite his publication. Nevertheless Maiden (1: 7) described Briosi's monograph as "a masterly paper." Knowledge thus won early has subsequently been forgotten.

We must ask if these findings extend to the other bluegum species.



Figures 1–4. All illustrations of leaves of *Eucalyptus globulus* from the publication by Briosi.(1892). **1** Stomata of a juvenile leaf. **2** Stomata of an adult leaf. **3** Section of a juvenile leaf with stomata only on the lower surface. **4** Section of a mature adult leaf.

Materials and Methods

Ausing cuticle preparations were obtained by chemical maceration in a heated mixture of glacial acetic acid and 100 vol. hydrogen peroxide (1:5), stained and mounted in glycerin-jelly. The measurements were made using a calibrated eyepiece micrometer and frequency measurements with an eyepiece graticule.

Observations

The observations (Tables 2–6) show that the juvenile leaves of *E st.johnii* (R.T.Baker) (a species subsumed by Kirkpatrick into his *E. pseudoglobulus*) and *E bicostata* Maiden also lack stomata on the upper surface and therefore have an internal structure like that of the juvenile leaves of *E. globulus*. But the juvenile leaves of the other species always have stomata on both surfaces, like the adult leaves of all. Thus, it matters not that such juvenile leaves are (as Briosi called them) "horizontal," the two surfaces illuminated unequally, and therefore (according to him) theoretically predisposed to be bifacial and have stomata confined to the lower surface whereas the vertically hanging adult leaves, as expected, are unifacial.

These facts stand, of course, in contrast to one of Kirkpatrick's statements (1974). "No discontinuities are evident in any of the 30 adult and juvenile characters studied over the total range of blue gum." The results reported below (Table 2) are quite preliminary and they must be taken as tentative and requiring confirmation and expansion. To go further with the comparisons would require a considerable research undertaking.

Table 2. *E. bicostata*, size of stomata (µm).

	Juvenile	Adult
Anzac Parade	33.5	55.8
Yarralumla	30.4	56.8

Ratio of adult/juvenile 1.78.

Of bluegum trees growing in Canberra *E. bicostata* is by far the commonest. In addition to cultivated trees of *E.st.johnii* I was also fortunate enough to have access to type material of that species collected by the eponymous E. St John from the Lerderderg Gorge in Victoria.

Table 3. *E. st johnii*. (= *E. pseudoglobulus* of Kirkpatrick) (lower surfaces)

	Juvenile	Adult
Lerderderg Gorge (E. St.John)	size 32.6µm freq. 266	57.4 µm 224.5
ANU campus site	36.58 freq. 187	46.17 —
near Mosque	size 39.68	54.7

The ratio of the (size) means) adult/juvenile is 1.47

As juvenile leaves of *E. maidenii* were not accessible I was forced to examine a number of herbarium specimens. One of these, *Beauglehole 33943*, had originally been identified as *E.st.johnii*, then was re-identified by Brooker as *E. pseudoglobulus* and subsequently used under that name as the voucher for a drawing of its three-flowered inflorescence in the Flora of Australia (Chippendale 1988). Its identification seems to me to be wrong and I believe that it is an unusual specimen of *E. maidenii*. For instance, the juvenile leaves have stomata on both surfaces like *E. maidenii* but unlike *E. pseudoglobulus*. The following are measurements of stomatal length in micrometres from the lower surfaces of the leaves.

Table 4. *E. maidenii*, size of stomata (lower sufaces)

	Juvenile	Adult
de Beuzeville 588 Eden NSW	29.05	58.7
de Beuzeville 587 Eden NSW	32.7	60.45
Beaglehole 33682 Genoa, Victoria	32.83	51.4
Beaglehole 33943 Tara Range, Vic.	28.163	62.46

The overall ratio of means of adult/juvenile sizes is 1.9. In some specimens (e.g. *Beauglehole 33943*) it is greater than 2.

Thus the ratios of the means of adult to juvenile sizes are as follows: *maidenii* 1.9, *globulus* 1.86, *bicostata* 1.67, *st.johnii* (= *peudoglobulus*) 1.47. Clearly the adult stomata of *E. maidenii* are by far the largest of any of the blue gums.

We are left with a puzzle: what are the so-called *E. globulus* specimens in and around Melbourne which superficially look like that species but have juvenile leaves with large numbers of stomata on the upper surface? They cannot be *E. pseudoglobulus* which lacks such stomata. The late J.H Willis also had difficulty with such specimens. "*E(ucalyptus) st.johnii* sometimes overlaps with occurrences of typical *E. globulus* (e.g. on Wilson's Promontory, Phillip Island and the Otways). where puzzling intermediate populations occur" (1973, 2: 419). Such so-called "intermediates" would include the specimen examined by Mueller (Table 1) and the Cameron specimen from Toorak. Evidently some trees identical with the real, Tasmanian, *E. globulus* exist in southern Victoria (like the one from Squeaky Bay, Wilsons Promontory), as one might expect since Tasmania has been isolated from the mainland only since the end of the last ice-age. But it is not possible immediately to classify the other specimens from the vicinity of Melbourne.

During one of my visits to California I collected a specimen of a locally grown variety (*E.globulus* var.*compacta* Maiden) near Santa Barbara. It is listed in Chippendale (1988) as a hybrid.

Table 5. *E. globulus* var. *compacta*. (size of stomata µm)

Juvenile	Adult
upper 39.76	upper 38.29
lower 33.18	lower 41.25
For comparison: Cameron Toorak (as "*E. globulus*")	
upper 26.18	upper 39.0
lower 33,32	lower 40

Ratios adult/juvenile (lower surfaces) compacta 1.2; Cameron Toorak 1.2)

The var. *compacta* ratio (Table 5) might suggest some relationship with *E pseudoglobulus*, some specimens of which (*e.g.* those on the ANU campus) have a ratio of 1.2, rather than with *E globulus*. However the fact that the juvenile leaves have stomata on both surfaces, unlike *E. pseudoglobulus*, does not support that possibility. The data added for the Toorak specimen of Cameron labelled '*E. globulus*' present a striking similarity except for the lengths of the stomata on the upper surfaces of the juveniles. Further work is suggested on the blue gums of Santa Barbara and those around Melbourne. Unfortunately the Santa Barbara tree appeared to be sterile, at least I saw on it no fruits or flowers.

Discussion

The ability to illuminate relationships within the bluegums by examining the leaf anatomy suggests that it, rather than characters such as the number of buds in the inflorescence, might be the better determinant. For instance, it is quite clear that stomatal sizes and distribution easily distinguish between *E. pseudoglobulus* and *E. maidenii* (as in the case of *Beauglehole 33943*).

A corollary of the measurements reported above, which include some measurements on juvenile foliage not from seedlings but from what are called "reversion shoots" on adult trees, is that the characters of the juvenile leaves are repeated when, in the production of these reversion shoots, the adult tree produces another set of juvenile leaves. These shoots usually begin with a few leaves, identical in shape and glaucousness to those of the sapling and to these characters we may now add those of the stomata. The sizes and locations of the stomata are identical with those of the seedling and sapling. Then as the reversion shoot develops, leaves identical with the adult leaves in all characters, including those of the stomata, are produced. This is quite a remarkable performance since it implies that a whole suite of juvenile characters, presumably all genetically determined and including cell size (since stomatal size appears so), is switched on and then in the course of the early development of the succeeding leaf primordia they are switched off and all replaced simultaneously by a different suite of adult characters, also genetically determined. Of course this performance merely repeats what occurs during normal "phase change" (juvenile to adult) during the normal growth of the tree, but in its recurrence and spontaneity it is very striking. During the slow development of the bark-enclosed residual meristem (which gives rise to the reversion shoot), in turn derived from an accessory bud on the growing shoot, there must be some reprogramming of the genetic material of the cells of the meristem to an embryonic state like that of the seedling; this state then changes rapidly during reversion shoot growth back to the adult condition (see Carr 1984).

In a previous publication (Carr & Carr 1991), we stated (without citing data) that "the size of the guard cells (of juvenile leaves of eucalypts) is smaller than those of the adult leaves. This I re-investigated in 7 species growing in my own garden or nearby in Canberra. The results are given below in Table 6.

Table 6. Comparisons of measurements of sizes of juvenile and adult stomata in some species of *Eucalyptus*.

Species ratio	adult/juvenile	significance
quadrangulata Deane & Maiden	1.220	$p< 0.05$
cinerea F Muell. *ex* Benth,	1.019	ns
bridgesiana R.T.Baker	1.136	ns
melliodora Cunn. *ex* Schauer.	1.205	ns
leucoxylon F.Muell.	1.1072	ns
sideroxylon Cunn. *ex* Woolls	1.1065	ns
perriniana F.Muell. *ex* Rodway	1.1535	$p< 0.001$

It will be clear that the results are equivocal. In all cases the measurements showed that the adult stomata were a little larger but the data when subjected to statistical test the differences were significant only in two cases (ns = not significant). The great differences in the bluegums appear therefore to be unique to that group of species. The number of stomata measured in each case was 30 for each surface and the tests were by t test. Perhaps if a larger sample of the stomata were measured in each case the differences observed might achieve significance. However, the differences are too small to warrant a fuller investigation.

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